

# Exhibit 5

# MOLECULAR MECHANISMS OF CANCER PAIN

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Pain is the most disruptive influence on the quality of life of cancer patients. Although significant advances are being made in cancer treatment and diagnosis, the basic neurobiology of cancer pain is poorly understood. New insights into these mechanisms are now arising from animal models, and have the potential to fundamentally change the way that cancer pain is controlled.

The negative impact that cancer pain has on the quality of life cannot be overestimated. As advances in cancer detection and therapy are extending the life expectancy of cancer patients, there is an increasing focus on improving patients' quality of life. For many patients, pain is the first sign of cancer, and 30–50% of all cancer patients will experience moderate to severe pain<sup>1–4</sup>. Cancer can cause pain at any time during the course of the disease, but the frequency and intensity of pain tend to increase during the advanced stages. In fact, 75–95% of patients with metastatic or advanced-stage cancer will experience significant amounts of cancer-induced pain<sup>1–4</sup>.

At present, a number of approaches are aimed at reducing the levels of cancer-related pain (TABLE 1). Therapies that aim to decrease tumour size are often effective and include radiation, chemotherapy and/or surgery — but these can be burdensome to administer and are accompanied by significant unwanted side effects. Moreover, medications that are targeted at decreasing inflammation-associated pain, such as non-steroidal anti-inflammatory drugs or opiates, also have many unwanted side effects. The relative ineffectiveness of current treatments reflects the fact that therapies have not changed for decades<sup>5–8</sup>. Largely because of treatment-associated side effects, it has been reported that 45% of cancer patients have inadequate and undermanaged pain control<sup>9,10</sup>. It has been a challenge to develop new approaches to relieve cancer-associated pain, as the neurobiological basis for pharmacological treatments is largely empirical and based on scientific studies of painful conditions other than cancer.

Recently, the first animal model of cancer pain was developed, involving injection of mouse osteolytic sarcoma cells into the intramedullary space of the mouse femur<sup>11</sup>. A crucial component of this model is that the tumour cells are confined to the marrow space of the injected femur and do not invade adjacent soft tissues<sup>11</sup>. After injection, the cancer cells proliferate, and both ongoing and movement-evoked pain-related behaviours increase as the tumour develops. These behaviours are correlated with the progressive tumour-induced bone destruction that ensues, and seem to mimic those of patients with primary or metastatic bone cancer<sup>12,13</sup>. This and other models have begun to provide insight into the mechanisms by which tumours cause pain and how this sensory information is processed. Insights such as these might lead to the development of new therapeutics that fundamentally change the way that cancer pain is controlled.

## Primary afferent sensory neurons

PRIMARY AFFERENT SENSORY NEURONS are the gateway by which sensory information from peripheral tissues is transmitted to the spinal cord and brain (FIG. 1). These neurons innervate the skin and almost every internal organ of the body. The cell bodies of sensory fibres that innervate the head and body are located in the TRIGEMINAL GANGLIA and DORSAL ROOT GANGLIA, respectively, and can be divided into two main categories: myelinated A-fibres and smaller-diameter, unmyelinated C-fibres. Nearly all large-diameter myelinated A-β fibres normally conduct non-noxious stimuli that are applied to the skin, joints and muscles. So, in a normal situation, these large sensory neurons do

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**PRIMARY AFFERENT SENSORY NEURON**

A neuron that has a cell body located in the dorsal root ganglion and has one axon that innervates peripheral tissue and one axon that projects to the spinal cord or the brainstem. Humans have 2–3 million primary afferent sensory neurons, which innervate almost every organ of the body.

**TRIGEMINAL GANGLIA**

The ganglia that house the cell bodies of primary afferent neurons that innervate the head and neck.

**DORSAL ROOT GANGLION**

The cell bodies of sensory neurons are collected together in paired ganglia that lie alongside the dorsal spinal cord. These sensory-neuron cell bodies are surrounded by satellite glial cells.

**NOCICEPTOR**

A primary afferent sensory neuron that is activated by tissue-damage-related stimuli.

**PURINERGIC RECEPTORS**

A family of cell-surface receptors that are activated by ATP and other nucleotides that mediate a broad spectrum of physiological responses, including activation of nociceptors.

**Table 1 | Therapies used to treat cancer pain**

Drug class	Mechanism of action	Effect	Indication	Complications
NSAIDs	COX1 and COX2 synthesis blockade	Analgesia, anti-inflammatory	Mild to moderate pain, bone pain, inflammation, enhance opioid effects	Bleeding, gastrointestinal ulceration, renal toxicity, ceiling effect
Opioids	$\mu$ -opioid receptor antagonism in the peripheral and central nervous systems	Analgesia	Moderate to severe pain	Constipation, nausea/vomiting, sedation, respiratory depression
Radiation	Destruction of dividing cells	Analgesia, tumour shrinkage	Primary therapy	Mucositis/pruritis, fatigue, nausea/vomiting
Systemic radioisotopes	Destruction of cells	Analgesia, tumour shrinkage	Therapy for bone metastasis	Myelosuppression
Corticosteroids	Multiple mechanisms	Analgesia, anti-inflammatory	Central nervous system oedema, spinal-cord and nerve or plexus compression, bone pain	Dysphoria, weight gain, dyspepsia, oropharyngeal candidiasis, diabetes mellitus, aseptic bone necrosis
Local anaesthetic	Blockade of sodium channels on peripheral nerves	Local anaesthesia	Cutaneous/mucosal pain, intrathecal application for severe pain	Short lasting, potential for tissue injury
Antidepressants	Inhibition of serotonin and NE re-uptake	Analgesia, mood modification	Neuropathic pain, musculoskeletal pain	Anticholinergic side effects, sedation, hypotension, cardiotoxicity seizures
Bisphosphonates	Cause apoptosis of osteoclasts in mineralized bone	Analgesia, tumour shrinkage, suppression of osteolysis	Lytic and blastic bone pain	Gastrointestinal tract toxicity, fever, electrolyte abnormalities

COX, cyclooxygenase; NE, norepinephrine; NSAIDs, non-steroidal anti-inflammatory drugs.

not conduct noxious stimuli<sup>14</sup>. By contrast, most small-diameter sensory fibres — unmyelinated C-fibres and finely myelinated A- $\delta$  fibres — are specialized sensory neurons that are known as **NOCICEPTORS**, the main function of which is to detect and convert environmental stimuli that are perceived as harmful

into electrochemical signals that are transmitted to the central nervous system. Unlike primary sensory neurons that are involved in vision or olfaction — which are required to detect only one type of sensory stimulus (light or chemical odorants, respectively) — individual primary sensory neurons of the pain pathway have the remarkable ability to detect a wide range of stimulus modalities, including those of a physical or chemical nature<sup>15,16</sup>. To accomplish this, nociceptors express a diverse repertoire of receptors and transduction molecules that can sense forms of noxious stimulation (thermal, mechanical and chemical) — albeit with varying degrees of sensitivity (FIG. 1).

In the past few years, remarkable progress has been made in understanding molecules that nociceptors use to detect noxious stimuli. For example, the vanilloid receptors (VR1), which are expressed by most nociceptors, detect heat<sup>17</sup> and also seem to detect acid<sup>18</sup>, extracellular protons<sup>19,20</sup> and lipid metabolites<sup>21,22</sup>. To detect noxious mechanical stimuli, nociceptors express both mechanically gated channels — which activate a signalling cascade in response to excessive stretching<sup>23</sup> — and several **PURINERGIC RECEPTORS**, which are activated by ATP. ATP is released from cells during excessive mechanical stimulation<sup>24,25</sup>. To sense noxious chemical stimuli, nociceptors express a complex array of receptors that are activated by inflammation-associated factors released from damaged tissue. These include protons<sup>19,20</sup>, **ENDOTHELINS**<sup>26</sup>, **PROSTAGLANDINS**<sup>27</sup>, **BRADYKININ**<sup>27</sup> and nerve growth factor<sup>28</sup>. Apart from providing promising targets for the development of more selective analgesics, the

**Summary**

- As advances in cancer detection and therapy are extending the life expectancy of cancer patients, there is increasing focus on improving the quality of life of patients. New approaches are desperately needed to control cancer-associated pain.
- Sensory information from peripheral tissues is transmitted to the spinal cord and brain by primary afferent sensory neurons. Specialized sensory neurons — known as **nociceptors** — detect and convert environmental stimuli that are perceived as harmful into electrochemical signals that are transmitted to the central nervous system.
- Tumours secrete a variety of factors that sensitize or directly excite primary afferent neurons, causing the sensation of pain. Receptors for many of these factors are expressed by primary afferent neurons.
- Both the intracellular and extracellular pH of solid tumours are lower than that of surrounding normal tissues, which can also activate sensory neurons and cause pain in cancer patients.
- Tumour growth entraps and injures nerves, causing neuropathic pain.
- The spinal cord and forebrain undergo neurochemical and structural changes as a state of chronic pain develops.
- Cancer pain frequently becomes more severe as the disease progresses, and might require different types of analgesic at different time points.
- For the first time, animal models of cancer pain are now available. These will offer insight into one of the main conundrums of cancer pain — why the severity of this pain is so variable from patient to patient, tumour to tumour, and even from site to site.

**ENDOTHELINS**

A family of three peptides that are released from endothelial cells and some tumour cells. These peptides can activate nociceptors, mount an inflammatory response, and stimulate angiogenesis and growth of tumour cells.

**PROSTAGLANDINS**

Pro-inflammatory lipids that are formed from arachidonic acid by the action of cyclooxygenase enzymes and other downstream synthetases.

**BRADYKININ**

A peptide that, when applied to primary afferent sensory nerve terminals, produces pain and sensitization of the sensory neuron to other noxious and non-noxious stimuli.

**PERIPHERAL SENSITIZATION**

An altered state of nociceptor function that is characterized by a lowered threshold of activation and an increased response to suprathreshold stimuli.

**HYPERALGESIA**

An increased response to a stimulus that is normally painful.

**ALLODYNIA**

Pain caused by a stimulus that does not normally provoke pain.

identification of receptors that are expressed on the surface of nociceptors has increased our understanding of how tumours generate pain as they invade and destroy peripheral tissues.

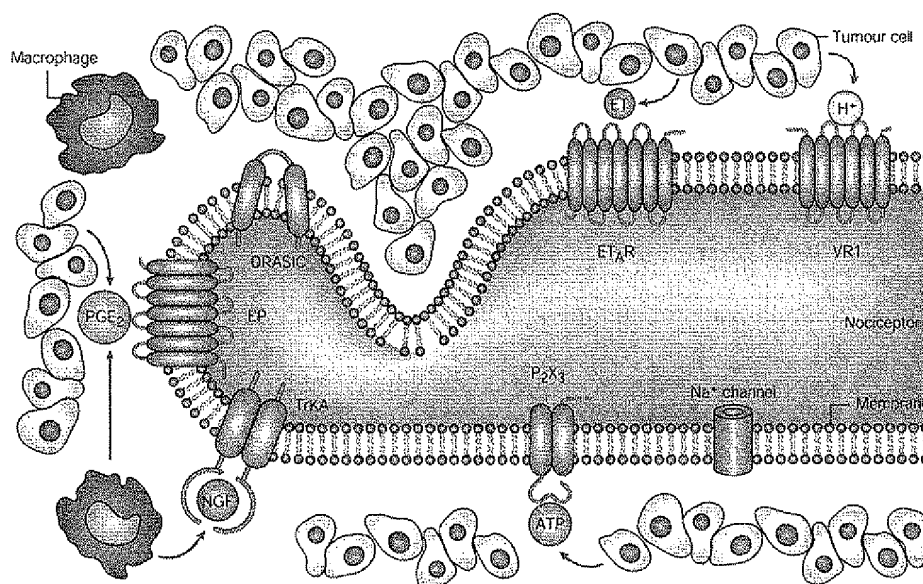
In addition to expressing channels and receptors that detect tissue injury, sensory neurons are highly 'plastic', in that they can change their phenotype in response to a sustained peripheral injury. After tissue injury, many nociceptors alter their patterns of signalling-peptide and growth-factor expression<sup>29</sup> (FIG. 2). This change in phenotype of the sensory neuron underlies, in part, **PERIPHERAL SENSITIZATION**, in which the nociceptor threshold level of activation is lowered, so that what would normally be perceived as a mild noxious stimulus is perceived as highly noxious (**HYPERALGESIA**), or stimuli that would normally be perceived as non-noxious are perceived as noxious (**ALLODYNIA**). Damage to a peripheral tissue has also been shown to activate previously 'silent' or 'sleeping' nociceptors, which then become highly responsive to normally non-noxious or mildly noxious stimuli<sup>30</sup>.

There are several examples of experimental cancer models in which peripheral nociceptors become sensitized<sup>11–13,31,32</sup>. In normal mice, the neurotransmitter substance P is synthesized by nociceptors and released in the spinal cord when noxious — but not non-noxious — mechanical stress is applied to the femur. In mice with bone cancer, what would normally be a

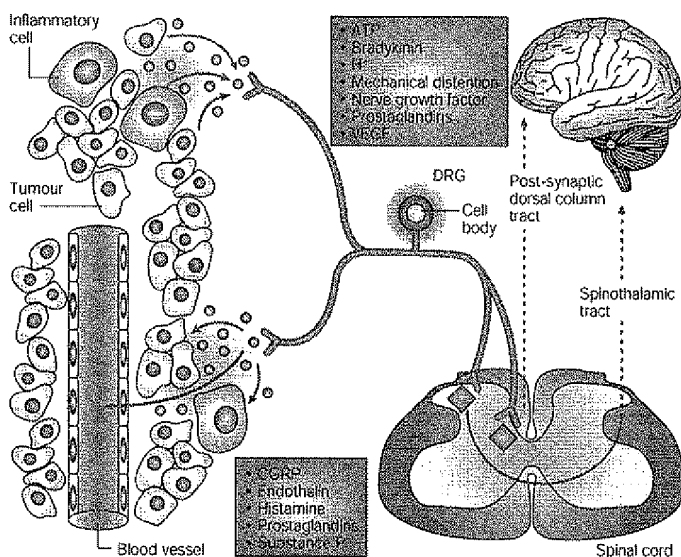
non-painful level of mechanical stress can induce the release of substance P from primary afferent fibres that terminate in the spinal cord. Substance P, in turn, binds to and activates the neurokinin-1 receptor, which is expressed by a subset of spinal-cord neurons<sup>33–35</sup>. Similarly, when what are normally non-noxious levels of mechanical stress are placed on the tumour-bearing limbs of mice with bone cancer, expression of the transcription factor c-Fos is induced in spinal-cord neurons. The expression of c-Fos in the superficial dorsal horn (laminae I–II) of the spinal cord has been used as a marker of activation of primary afferent terminals<sup>36–39</sup>, and sensitization of terminals in inflammatory<sup>40,41</sup> and sarcoma-induced bone cancer pain<sup>11–13,41</sup> states. In animals that do not have cancer, only noxious stimuli induce the expression of c-Fos in the spinal cord<sup>39</sup>. So, peripheral sensitization of nociceptors is involved in the generation and maintenance of bone cancer pain.

**Nociceptor stimulation by tumours**

A tumour is made up of many cell types other than cancer cells, including immune-system cells such as macrophages, neutrophils and T cells. These secrete various factors that sensitize or directly excite primary afferent neurons (FIG. 2), and include prostaglandins<sup>42,43</sup>, tumour necrosis factor- $\alpha$  (TNF- $\alpha$ )<sup>44,47</sup>, endothelins<sup>26,48</sup>,



**Figure 1 | Detection by sensory neurons of noxious stimuli produced by tumours.** Nociceptors (pink) use several different types of receptor to detect and transmit signals about noxious stimuli that are produced by cancer cells (yellow) or other aspects of the tumour microenvironment. The vanilloid receptor-1 (VR1) detects extracellular protons ( $H^+$ ) that are produced by cancer cells, whereas endothelin-A receptors ( $ET_A R$ ) detect endothelins (ET) that are released by cancer cells. The dorsal-root acid-sensing ion channel (DRASIC) detects mechanical stimuli as tumour growth mechanically distends sensory fibres. Other receptors that are expressed by sensory neurons include prostaglandin receptors (EP), which detect prostaglandin  $E_2$  ( $PGE_2$ ) that is produced by cancer and inflammatory cells (macrophages). Nerve growth factor (NGF) released by macrophages binds to the tyrosine kinase receptor TrkA, whereas extracellular ATP binds to the purinergic  $P_2X_2$  receptor. Activation of these receptors increases the excitability of the nociceptor, inducing the phosphorylation of the 1.8 and/or 1.9 sodium channel ( $Na^+$  channel) and decreasing the threshold required for nociceptor excitation. Figure adapted from REF. 16.



**Figure 2 | The tumour-nociceptor interface.** In addition to cancer cells, tumours consist of inflammatory cells and blood vessels, and are often adjacent to primary afferent nociceptors. Cancer cells and inflammatory cells release a variety of products, such as ATP, bradykinin,  $H^+$ , nerve growth factor, prostaglandins and vascular endothelial growth factor (VEGF), that either excite or sensitize the nociceptor. Painful stimuli are detected by the nociceptors, the cell bodies of which lie in the dorsal root ganglion (DRG), and are transmitted to neurons in the spinal cord. The signal is then transmitted to higher centres of the brain. Cancer-associated pain signals seem to ascend to the brain by at least two main spinal-cord pathways — the spinothalamic tract and the dorsal column. Nociceptor activation results in the release of neurotransmitters, such as calcitonin gene-related peptide (CGRP), endothelin, histamine, glutamate and substance P. Nociceptor activation also causes the release of prostaglandins from the peripheral terminals of sensory fibres, which can induce plasma extravasation, recruitment and activation of immune cells, and vasodilatation. Figure adapted from REFS 121, 122.

interleukin-1 and -6 (REFS 44,49,50), epidermal growth factor<sup>51</sup>, transforming growth factor- $\beta$ <sup>52,53</sup> and platelet-derived growth factor<sup>54,56</sup>. Receptors for many of these factors are expressed by primary afferent neurons. Although each of these factors might be important in generating pain in particular forms of cancer, drugs that target prostaglandins and endothelins are the only ones used, at present, to control pain in cancer patients.

Prostaglandins are pro-inflammatory lipids that are formed by the action of cyclooxygenase (COX) enzymes and other downstream prostaglandin synthetases. Prostaglandins are involved in the sensitization and/or direct excitation of nociceptors by binding to several prostanoid receptors that are expressed by nociceptors<sup>57</sup>. Two separate COX isoforms are involved in prostaglandin synthesis. COX1 is constitutively expressed by most tissues, whereas COX2 is expressed only under inflammatory conditions. Cancer cells and tumour-associated macrophages express high levels of COX2, leading to high levels of prostaglandin production<sup>58,62</sup>.

Drugs such as aspirin or ibuprofen — which are commonly administered to reduce both inflammation and pain — inhibit both COX enzymes. A significant problem with using mixed COX1 and COX2 inhibitors to block cancer pain is that COX1 maintains the normal

gastric mucosa, and its inhibition can cause bleeding and ulcers. COX2-specific inhibitors, by contrast, do not cause gastrointestinal-tract complications. COX2 has also been associated with angiogenesis and tumour growth<sup>63,64</sup>, so in addition to blocking cancer pain, COX2 inhibitors might also slow cancer progression. COX2 antagonists show significant promise for alleviating at least some aspects of cancer pain, although clearly more research is required to fully define the actions of COX2 in different types of cancer<sup>65,66</sup>.

Endothelin antagonists are a second type of pharmacological agent that might be useful in reducing pain sensation. Endothelins (endothelin-1, -2 and -3) are a family of vasoactive peptides that are expressed at high levels by several types of tumour, including prostate cancer<sup>66,67,68</sup>. Clinical studies have shown a correlation between the severity of pain and plasma levels of endothelins in prostate cancer patients<sup>69</sup>. Endothelins could contribute to cancer pain by directly sensitizing or exciting nociceptors, as a subset of small, unmyelinated primary afferent neurons expresses endothelin-A receptors<sup>70</sup>. Furthermore, direct application of endothelin to peripheral nerves induces activation of primary afferent fibres and an induction of pain behaviours<sup>71</sup>. Like prostaglandins, endothelins that are produced by cancer cells are also thought to be involved in regulating angiogenesis<sup>72</sup> and tumour growth<sup>73</sup>. These findings indicate that endothelin antagonists might be useful not only in inhibiting cancer pain, but also in reducing tumour growth and metastasis.

**Tumour-induced acidosis.** Both the intracellular and extracellular pH of solid tumours are lower than that of surrounding normal tissues<sup>74</sup>. Local acidosis — characterized by the accumulation of acid metabolites — is a hallmark of tissue injury<sup>16,75</sup>. The finding that sensory neurons can be directly excited by protons or acid has generated intense interest in this subject among basic and clinical researchers<sup>76</sup>. Studies have shown that subsets of sensory neurons express different acid-sensing ion channels<sup>16,77</sup>. The two main classes of acid-sensing ion channels that are expressed by nociceptors are VR1 (REFS 78,79) and the acid-sensing ion channel-3 (ASIC3)<sup>76,77,80</sup>. Both of these channels are sensitized and excited by a decrease in pH. More specifically, VR1 is activated when the pH falls below 6.0, whereas the pH that activates ASIC3 seems to be highly dependent on the co-expression of other ASIC channels in the same nociceptor<sup>81</sup>.

There are several mechanisms by which tumours could cause a decrease in pH. As inflammatory cells invade the neoplastic tissue, they release protons that generate local acidosis. The large amount of apoptosis that occurs in the tumour environment also contributes to acidosis, as apoptotic cells release intracellular ions to create an acidic environment. This drop in pH can activate signalling by acid-sensing channels that are expressed by nociceptors.

Tumour-induced release of protons and acidosis might be particularly important in the generation of bone cancer pain. Both osteolytic (bone-destroying) and osteoblastic (bone-forming) cancers are characterized by

Table 2 | Mechanism-based therapies under development that target specific components of bone cancer pain

Drug class	Target	Action	Indication	Potential complication
Osteoprotegerin	Osteoclast activation	Inhibits osteolysis	Bone cancer pain	Alterations in bone metabolism
Selective COX2 inhibitors	Prostaglandin synthesis, bone cells	Prevent sensitization of nerve fibres by prostaglandins, suppress tumour growth	Prostaglandin-dependent cancers	Nephrotoxicity, cardiotoxicity, gastrointestinal complications (although reduced incidence compared with NSAIDs)
Endothelin-receptor antagonists	Nerve fibres, smooth-muscle cells	Prevent sensitization of nerve fibres by endothelin	Endothelin-sensitive cancers	Hypotension, teratogenicity
VR1 antagonists	pH-sensitive nerve fibres	Blockade of protons through VR1 channels on nerve fibres	Proton- or acid-producing cancers	Delayed wound healing, altered taste perception
Purinergic-receptor antagonists	ATP-sensitive nerve fibres	Blockade of ATP by P <sub>2</sub> receptors on nerve fibres	Cancers that invade mechanically sensitive tissues	Altered touch perception, urinary retention
Anticonvulsants (gabapentin (Neurontin); carbamazepine)	Calcium channel subunit, NMDA-receptor subunit, sodium channels	Suppress aberrant neuronal discharges	Cancers with neuropathic component	Bone-marrow suppression, drowsiness, ataxia, oedema
ASIC blockers	pH-sensitive nerve fibres	Blockade of protons through ASICs on nerve fibres	Proton- or acid-producing cancers	CNS-related side effects due to wide distribution in CNS
Substance P (saporin)	Spinal-cord neurons that express the substance P receptor	Destruction of neurons that are involved in conducting pain	Intractable cancer pain	Permanent destruction of neurons

ASIC, acid-sensing ion channel; COX, cyclooxygenase; CNS, central nervous system; NMDA, *N*-methyl-D-aspartate; NSAID, non-steroidal anti-inflammatory drug; VR1, vanilloid receptor-1.

osteoclast proliferation and hypertrophy<sup>82–84</sup>. Osteoclasts are terminally differentiated, multinucleated, monocyte-lineage cells that resorb bone by maintaining an extracellular microenvironment of low pH (4.0–5.0) at the osteoblast-mineralized bone interface<sup>85</sup>. Most sensory neurons that innervate bone express VR1 (REF. 86) and/or ASICs<sup>77</sup>, so these sensory neurons become depolarized and transmit pain signals to the spinal cord when exposed to the osteoclasts' acidic extracellular microenvironment. Recent experiments in a mouse model of bone cancer pain reported that osteoclasts have an essential role in cancer-induced bone loss and also contribute to the aetiology of bone cancer pain<sup>12,13</sup>.

VR1 or ASIC antagonists might therefore be useful in reducing pain in patients with soft or bony tumours, by blocking excitation of the acid-sensitive channels on sensory neurons. Antagonists for these channels are now being developed. Drugs known as bisphosphonates, which induce osteoclast apoptosis, have also been reported to reduce pain in patients with osteoclast-induced skeletal metastases<sup>87–89</sup>. Bisphosphonates are pyrophosphate analogues that display high affinity for calcium ions, causing them to rapidly target the mineralized matrix of bone<sup>90</sup>. These drugs have been reported to act directly on osteoclasts, inducing their apoptosis by impairing the synthesis of either ATP or cholesterol — both of which are necessary for cell survival<sup>91,92</sup>. Osteoclasts that have been treated with bisphosphonates undergo morphological changes, including cell shrinkage, chromatin condensation, nuclear fragmentation and loss of the ruffled border — all of which are signs of apoptosis<sup>90</sup>. Studies in both clinical<sup>87–89</sup> and animal<sup>93–95</sup> models of bone cancer have reported anti-resorptive effects of bisphosphonate therapy, although the effects on long-term survival rates and tumour growth remain controversial.

Osteoprotegerin (OPG) is another agent that holds significant promise for alleviating bone cancer pain (TABLE 2). OPG is a secreted soluble receptor that is a member of the tumour necrosis factor receptor (TNFR) family<sup>96</sup>. This receptor prevents the activation and proliferation of osteoclasts by binding to and sequestering osteoprotegerin ligand (OPGL; also known as the receptor for activator of NF- $\kappa$ B ligand, RANKL)<sup>97,98</sup>. The activation and differentiation of osteoclasts requires the interaction of the receptor for activator of NF- $\kappa$ B (RANK), which is expressed on osteoclast precursors, with OPGL/RANKL, which is expressed on osteoblasts<sup>99</sup>. Sequestration of OPGL by OPG prevents the binding of OPGL/RANKL to RANK, which, in turn, prevents osteoclast differentiation and activation<sup>92,99</sup>. Although OPG has been shown to decrease pain behaviours in an animal model of bone cancer<sup>12,13</sup>, it is still being developed for use in cancer patients. OPG has also been shown to increase bone-mineral density and bone volume, which are associated with a decrease in active osteoclast number in women with osteoporosis<sup>100</sup>.

#### Tumour-induced distention of sensory fibres

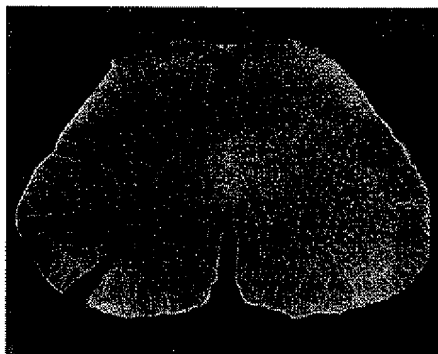
Tumours are not highly innervated by sensory neurons<sup>101–103</sup>. Rapid tumour growth frequently entraps and injures nerves, however, causing mechanical injury, compression, ischaemia or direct proteolysis<sup>1</sup>. Proteolytic enzymes that are produced by the tumour cells can also cause injury to sensory and sympathetic fibres, causing neuropathic pain. The capacity of tumour cells to injure and destroy peripheral nerve fibres has been directly observed in an experimental model of bone cancer (P. W. M., unpublished observations). After injection and containment of lytic sarcoma cells in the intramedullary canal of the mouse femur,

## ASTROCYTES

The most numerous type of glial cell in the central nervous system. Astrocytes regulate the extracellular neuronal environment.

## CENTRAL SENSITIZATION

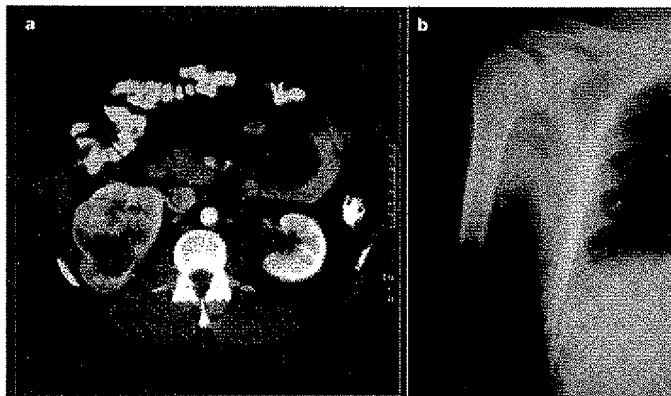
An increased responsiveness of pain transmission to neurons in the spinal cord — usually caused by neurochemical changes in the spinal cord, brainstem or forebrain.



**Figure 3 | Cancer induced reorganization of the central nervous system.** Chronic cancer pain can cause significant alterations in the central nervous system. This is believed to underlie the phenomenon of central sensitization — an increased responsiveness of spinal-cord neurons that are involved in the transmission of pain. Confocal imaging of glial fibrillary acidic protein (GFAP) expression in the L4 segment of a tumour-bearing mouse spinal cord shows an increase in the number of astrocytes on the right side of the spinal cord, which receives sensory innervation from the tumour-bearing bone. The left side of the spinal cord, which is not transmitting painful stimuli to the brain, has fewer astrocytes.

## Box 1 | The conundrum of cancer pain

On the basis of clinical observations, pain from cancer can seem quite perplexing because the size, location or type of cancer does not necessarily predict symptoms. Different patients with the same cancer can experience differing amounts of pain. Kidney cancer might be painful in one person and asymptomatic in another. Different bone metastases in the same individual might cause pain in a rib bone, but not in the humerus. Small cancer deposits in bone might be painful, whereas large soft-tissue cancers might be painless. For example, in panel a, a computerized tomographic image of a patient reveals a large tumour of the kidney, although this patient reported no pain; in panel b, a different patient, who had a bony metastasis of similar size that arose from lung, reported severe pain. So, from the clinical perspective, the size of the lesion or tumour mass does not dictate or correlate with the quality and severity of pain, and might in some ways explain the heterogeneity of human cancer pain. Identifying the molecular mechanisms that underlie the various aspects of cancer pain — such as site specificity, differences between soft-tissue- and bone-tumour-induced pain, and the causes of different types of pain induced by tumours in different types of bone — is one of the primary objectives of pain researchers.



tumour cells grow in the marrow space, which is also innervated by sensory fibres. As the tumour cells grow, they first compress and then destroy both the haematopoietic cells that normally comprise the marrow, and the sensory fibres that normally innervate the marrow and mineralized bone.

Although the mechanisms by which neuropathic pain is generated and maintained are still not well understood, several therapies have proved useful in the control of non-cancer-induced neuropathic pain. For example, gabapentin, which was originally developed as an anticonvulsant, but has an unknown mechanism of action, is effective in treating several forms of neuropathic pain, and might be useful in treating cancer pain of neuropathic origin<sup>101</sup>.

There is ample evidence to support the contention that tumour cells themselves — along with tumour-associated inflammatory cells — have a significant role in the generation of cancer pain. Nonetheless, therapies that are aimed at tumour-cell eradication (such as chemotherapeutic agents) also cause significant levels of pain in cancer patients<sup>105</sup>. Potential mechanisms by which chemotherapeutic agents (such as paclitaxel and vincristine) cause peripheral neuropathy include their ability to disrupt tubulin function. Tubulin polymerization is necessary for axonal transport of trophic factors, and drugs that interfere with this process can cause degeneration of sensory neurons and release of pro-inflammatory cytokines that directly sensitize primary afferent nociceptors<sup>106,107</sup>. Understanding the mechanisms by which tumours and chemotherapeutic agents cause cancer pain might lead to the discovery of pharmacological agents that are used for non-malignant conditions, but that can also be used to reduce cancer pain, thereby eliminating these types of side effect.

## Central sensitization in cancer pain

A crucial question is whether the spinal cord and forebrain also undergo neurochemical changes as a state of chronic pain develops. Studies involving the mouse model of bone cancer pain described above showed extensive neurochemical reorganization in the spinal-cord segments that receive input from primary afferent neurons that innervated the tumour-bearing bone<sup>12,13,41</sup> (FIG. 3). This includes an astrocyte hypertrophy, which is accompanied by a decreased expression of glutamate re-uptake transporters<sup>108,109</sup>. This results in increased extracellular levels of the excitatory neurotransmitter glutamate and concomitant excitotoxicity within the central nervous system. The upregulation of the pro-hyperalgesic peptide dynorphin was also observed in the spinal cords of tumour-bearing animals. Spinal-cord expression of dynorphin — a pro-nociceptive member of the opioid family<sup>110–112</sup> — has been observed in animal models of neuropathic<sup>113,114</sup>, inflammatory<sup>115–118</sup> and sarcoma-induced bone cancer<sup>11–13,41</sup> pain states. As a result, spinal-cord neurons that would normally be activated only by noxious stimuli can be activated by stimuli that would normally be non-noxious. These changes can be

**SPINOTHALAMIC TRACT NEURONS**

A small group of neurons that are located in the dorsal horn of the spinal cord and are involved in the ascending conduction of pain and temperature.

**AMYGDALA**

An area of the forebrain that is involved in the formation of emotional memories, and the generation of fear, anxiety and stress that occurs in response to noxious stimuli.

**PERIOSTEUM**

The thin, highly innervated, fibrous tissue sheath that covers the outside of mineralized bone.

attenuated by blocking tumour-induced tissue destruction and pain<sup>12,13</sup>. Cancer pain therefore induces, and is at least partially maintained by, a state of CENTRAL SENSITIZATION, in which neurochemical changes in the spinal cord and forebrain promote an increased transmission of nociceptive information.

Once nociceptive information has been transmitted to the spinal cord by primary afferent neurons, there are many ascending 'pain' pathways that project from the spinal cord to higher centres of the brain. Classically, the main emphasis in examining the ascending conduction of pain has been placed on SPINOTHALAMIC TRACT NEURONS. Clinical studies, however, have necessitated a reassessment of this position, reporting that attenuation of some forms of visceral cancer pain can be achieved by disrupting non-spinothalamic-tract axons<sup>119,120</sup>. One reason, therefore, that cancer pain is perceived as so intense and disturbing is that it is transmitted to the brain by means of several parallel neuronal pathways. Importantly for cancer patients, it is clear that higher centres of the brain — such as the AMYGDALA and cerebral cortex — can modulate the ascending conduction of pain. This means that the general mood and attitude of the patient might also be a significant factor in determining the intensity and degree of pain.

**Different stages and types of cancer pain**

Cancer pain frequently becomes more severe as the disease progresses, and might require different types of analgesic at different time points. In the mouse model of bone cancer, for example, pain-related behaviours are present before any significant bone destruction is evident<sup>13</sup>. This pain might be caused by pro-hyperalgesic factors, such as prostaglandins and endothelin, which are released by the cancer cells that activate nociceptors in the bone marrow. COX2 inhibitors and endothelin antagonists could be used to relieve pain at this stage. As the tumour continues to grow, sensory neurons that innervate the marrow are compressed and destroyed, causing neuropathic pain that might respond to treatment with drugs such as gabapentin. As the tumour induces proliferation and hypertrophy of osteoclasts, pain caused by osteoclast

activity could be blocked with anti-osteoclastogenic drugs such as bisphosphonates or osteoprotegerin. Then, as the cancer cells completely fill the intramedullary space, the high levels of resulting apoptosis generate an acidic environment. In this situation, antagonists to VR1 or ASICs could be used to attenuate the pain that is induced by acidosis. Finally, as bone destruction continues and the mechanical strength of the bone is compromised, antagonists that block the mechanically gated channels and/or ATP receptors in the richly innervated PERIOSTEUM might reduce movement-associated pain.

Although this pattern of tumour-induced tissue destruction and nociceptor activation might be unique to bone cancer, an evolving set of nociceptive events probably occurs in other cancers. This might, in part, explain why cancer pain is frequently difficult to treat and why it can be so heterogeneous in nature and severity. Because the type of tumour-induced tissue injury, level of nociceptor activation, and the spinal cord and forebrain areas involved in transmitting nociceptive signals change as the disease progresses, different therapies might be more efficacious at particular stages of the disease.

**Future directions**

For the first time, animal models of cancer pain are now available that begin to mirror the clinical picture of humans with cancer pain. Data generated from these models will provide important new information about the mechanisms that generate and maintain the different types of cancer pain. These animal models might also offer insight into one of the main conundrums of cancer pain — why the severity of cancer pain is so variable from patient to patient, tumour to tumour, and even from site to site (BOX 1). Microarray and proteomic analyses will provide more information about specific features of tumours that cause cancer pain. Ultimately, gaining insight into the exact mechanisms by which different types of cancers excite nociceptors — and how the phenotype of nociceptors and central nervous system neurons that are involved in nociceptive transmission change as the disease progresses — will lead to the design of specific and effective analgesics for cancer pain (TABLE 2).

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# Online links

## DATABASES

The following terms in this article are linked online to: CancerNet (<http://www.cancer.gov/search/>) bone cancer | prostate cancer  
LocusLink: <http://www.ncbi.nlm.nih.gov/LocusLink/> ASIC3 | COX1 | COX2 | endothelin-1 | endothelin-2 | endothelin-3 | epidermal growth factor | c-Fos | interleukin-1 | interleukin-6 | nerve growth factor | neurokinin-1 receptor | OPC | OPGL | P<sub>2</sub>X<sub>1</sub> | platelet-derived growth factor | RANK | TNFR | TNF- $\alpha$  | transforming growth factor- $\beta$  | tubulin | VRI  
Medscape DrugInfo: <http://promini.medscape.com/drugdb/search.asp> aspirin | gabapentin | ibuprofen | paclitaxel | vincristine

## FURTHER INFORMATION

American Alliance of Cancer Pain Initiatives: <http://www.aacpi.org/>  
American Cancer Society: <http://www.cancer.org/>  
American Pain Foundation: <http://www.painfoundation.org/>  
American Pain Society: <http://www.ampainsoc.org/>  
Evidence-Based Health Care, Oxford: <http://www.j2.ox.ac.uk/bandolier/>  
International Association for the Study of Pain (IASP): <http://www.iaicyon.com/>  
National Cancer Institute: <http://www.nci.nih.gov/>  
National Institute of Drug Abuse: <http://www.nida.nih.gov/>  
National Institute of Neurological Disorders and Stroke: <http://www.ninds.nih.gov/>  
Neurosystems Laboratory: <http://neurosystems.umn.edu/>  
NIH Clinical Trials Listing: <http://clinicaltrials.gov/ct/gui/>  
Society for Neuroscience: <http://www.sfn.org/>  
University of Minnesota Cancer Center: <http://www.cancer.umn.edu/>  
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